

PART II

Conjugational junctions: The specific cell to cell contacts in bacterial conjugation.

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ABSTRACT

Electron micrographs of sections of mating pairs of bacteria that were arranged flat and parallel to the plane of slicing allowed us to observe a statistically significant number of conjugational junctions. These are specific for the F-mediated contacts and are not found in contacts between the same strains but lacking the F-plasmid. The conjugational junction is already found in mutants in which the DNA-transfer is inhibited. Upon transfer no visible modification of the junction occurs. Serial sections and tilt experiments exclude a plasma bridge with membrane fusions. The techniques used do not allow to observe eventually occurring pores of a size estimated to be below some 20 nm.

## INTRODUCTION

Visualisation in the electron microscope of conjugating bacteria by thin sections is associated with statistical and technical problems. In a conjugation mixture at any given moment, only one to ten out of a thousand mating pairs are actually transferring DNA. In addition, they are randomly oriented in space, so that the chance is very low to see a real contact in a conventionally made thin section. The above consideration, paired with the relatively poor structural preservation of membranes with conventional procedures led to controversial observations in the past. Gross and Caro (1966) found that mating pairs had - in their nomenclature - a "bridge of about 0.1  $\mu$ m in diameter of the two cell walls in a close juxtaposition". In text books frequently "real fusions" between the cytoplasms of two cells were given as representing conjugation. By the fusion tube, cells were kept at a distance of some 1000 Å. Schreil and Christensen (1968) found "conjugation bubbles" between donor and recipient cells. Under the light microscope Ou and Anderson (1970) separated individual mating pairs with a micromanipulator and observed them during mating. Some cells, according to the observations of these authors, were never in close wall to wall contact during mating, but supposed to be connected by pili and in fixed relative positions. After 30 min the recipients could be separated and analysed as  $F^+$  transconjugants. This result raised the model of DNA transfer through the lumen of pili (Brinton, 1971).

Curtis (1969) and Marvin and Hohn (1969) proposed that F-pili act as contact mediators between donor and recipient and are pulling the cells together by retraction. Achtman et al. (1971) started intensive genetic studies on the involvement of the single F-plasmid coded genes in the conjugational DNA transfer in E. coli. The results out of it have recently been reviewed by Willets and Wilkins (1984) and by Ippen-Ihler and Minkley (1986). Genetic data suggest that conjugation is proceeding through an ordered series of functional steps, each based on the expression of specific tra genes. Mutants in tra A, L, E, K, B, V, W, C, U, F, H and in one moiety of G do not produce pili and conjugate with highly reduced frequency. Donor mutants in tra N or in the other moiety of tra G and recipient mutants in ompA form shear sensitive (unstable) mating pairs that transfer DNA with reduced frequency. Mutants in tra M, D, I, Z are pilated and form shear resistant (stable) mating pairs that are deficient in the DNA transfer associated metabolism. As a consequence of these genetic studies, a sequential model mechanism for DNA transfer has been proposed by Willets (1980) that contained a step of stable wall to wall contacts and is consistent with most data available.

In a more recent review by Willets and Wilkins (1984) an improved version of the model for DNA transfer was published that now accounts for all genetic data. It assumes a plasma bridge with a fusion of inner and outer membrane between the mating partners and tra D protein as a pore former. Panicker

and Minkley (1985) demonstrated that the step of stabilised wall to wall contact is a defined intermediate in conjugational DNA-transfer.

Our approach consisted in designing experiments such that the number of conjugational contacts was so high that the observable number on micrographs was statistically significant and not only a rarely occurring situation. To achieve this goal, we deposited preformed mating pairs flat on an agar surface. Donor and recipient cells were morphologically different so as to enable the distinction of only parental pairs. We chose adequate mutants to achieve that more than 50% of the parental cells were associated in pairs. We used a donor mutant which was arrested in DNA transfer, so as to observe the contacts before DNA-transfer was initiated. In another parental combination we were able to produce pairs of which 60% were in the process of transferring DNA. These statistically sufficiently high numbers allowed us to observe a very large proportion of mating pairs which showed the specific conjugational junctions. On single micrographs it was possible to see several such junctions.

## MATERIALS AND METHODS

### Materials

M9 minimal medium was made by mixing 100 ml salt mixture, 100 ml 4% glucose, 10 ml 0.01 M  $\text{CaCl}_2$ , 10 ml 0.1 M  $\text{MgSO}_4$ , 0.2 ml ferric citrate solution with 780 ml  $\text{H}_2\text{O}$ . Salt mixture is 393 mM  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ , 220 mM  $\text{KH}_2\text{PO}_4$ , 86 mM NaCl and 187 mM  $\text{NH}_4\text{Cl}$ . M9 plates were made by adding 15 g Bacto agar Difco. The selective plates for hfr mapping were supplemented after autoclaving with 1 ml/l of a sterile-filtered 20% solution of the amino acids of interest. Tryptone broth contains 10 g Bacto tryptone Difco and 5 g NaCl in one l.  $\text{H}_2\text{O}$ . For Tryptone top (or soft) Agar 7 g per liter Bacto agar Difco were added. Filtration agar plates contain 10 g Bacto tryptone Difco, 5 g NaCl and 15 g ash-free Nobel agar in 1333 ml  $\text{H}_2\text{O}$ . The medium is autoclaved and 30 ml were poured in petri dishes (Ø 90 mm). Plates were left at room temperature until the medium had lost 30% of its initial weight by evaporation (up to ten days). Plates prepared this way were able to suck up 5 ml Tryptone broth in 30 to 60 minutes.

McConkey lac plates contain 20 g peptone, 1.5 g bile salt (Bacto No. 3), 5 g NaCl, 13.5 g Bacto agar Difco and 7 ml 1% neutral red solution (Bacto) in 993 ml  $\text{H}_2\text{O}$ . After autoclaving 30 ml of the above solution were poured in petri dishes (Ø 90 mm). Selective media were prepared by adding either 30 µg/ml spectinomycin or 10 µg/ml streptomycin or 30 µg/ml kanamycin to the liquid media before pouring.

### Strains and plasmids

The strains used are listed in Table I. All strains and plasmids marked with JC were from the collection of Achtman et al. (1971). The E. coli Cl22 is from the British Culture Collection and has been chosen for electron microscopical experiments because of its ovoid form and its peripheral fragmented nuclear region which was different from the normal aspect of nuclear material (Lieb et al., 1954; Gros and Caro, 1966). A lac<sup>-</sup> derivative has been made by UV light induced mutagenesis. Hfr1 has been constructed as shown in flow chart I. From ten transferring constructs the best was selected, named Hfr1 and mapped by mating with AB1157:pBR325 (Howard and Theriot, 1966) and separate marker transfer analysis.

### Matings for efficiency determination

Donor and recipient cultures were grown in 10 ml trypton broth on a rotating wheel for aeration at the temperature needed to a cell density of  $4 \times 10^8$  per ml. 1 ml of donor and recipient each were mixed in a prewarmed 250 ml Erlenmayer flask. The mixtures were incubated without shaking in a thermostatised chamber. Every 15 minutes an aliquot of 0.05 ml was taken, diluted 100x and heavily vortexed for 60 seconds. The samples were further diluted in phosphate buffer and analysed: a) for recombinant recipients having received the lac marker, b) with an antibiotic selective for donors and c) for prototroph recombinant recipients. The total titer was determined on nonselective McConkey lac plates.

### Preparing monolayers

Donor and recipient bacteria were grown in 10 ml tryptone broth on a rotating wheel at the temperature needed to a cell density of  $4 \times 10^8$ /ml. 2.5 ml of each were mixed in a prewarmed Erlenmayer flask and then poured on a prewarmed 30% predried filtration agar plate (initial volume 30 ml, 90 mm diameter) carrying a paper disc prepared as shown in Fig. 1. The cigarette paper disc (Job No. 8075, diameter 6 mm) had been immersed into hot tryptone top agar (90°C) and immediately placed on the surface of the filtration agar plate to get a thin and flat agar surface. After 30 min incubation with the mating mixture, the liquid was sucked up into the plates. Matings with Hfr1 as donor were further incubated at 30°C up to 150 min. Matings with JC6296 as donor were further incubated at 37°C up to 90 min. The density and quality of the monolayer was checked from identically prepared plates in the phase contrast light microscope.

### Cryofixation and embedding

The specimen on its support was mounted in tweezers and immersed into liquid propane at -190°C with a speed of 4m/sec over a distance of 70 mm. After a short storage in liquid nitrogen, the specimen was placed for 64 h at -90°C in a substitution medium containing 1% OsO<sub>4</sub> in acetone in presence of molecular sieve to adsorb water completely (0.4 nm perlform; Merck). The temperature was raised stepwise and the sample was embedded in Epon as described by Hobot et al. (1984). For polymerisation samples were put with the monolayer side down onto the flat bottom of polyethen capsules

(Balzers Union No. 05066). Polymerised like that, the monolayer is preoriented for horizontal sectioning in a microtome. If it has to be sectioned vertically, the monolayer was cut out with a saw and glued on an aluminium rod with an angle of  $90^{\circ}$  in a special device. Thin sections were cut with a diamond knife on an LKB Ultramicrotome III, stained with 4% aqueous uranyl acetate and lead citrate (Reynolds, 1963). Sections were examined in a Philips 300 electron microscope at 80 kV.

## RESULTS

### Transfer efficiencies in 1:1 mating mixtures

For looking at monolayers of conjugating bacteria in the electron microscope it is necessary to mix donor and recipient each in equal proportions. The kinetics of the different 1:1 mixtures used for electron microscopy was followed to establish conditions where electron micrographs could show more than 50% of the expected mating specific contacts. As recipient, the ovoid Cl22 strain (with peripheral "fragmented" nucleoid) was used (Lieb et al., 1955) because it can be distinguished from the rod shaped donors in the electron microscope. Cl22 has a normal amount of ompA, as was determined by SDS-polyacrylamid gel electrophoresis. It has also heptose containing LPS, as was determined by specific phage adsorption tests (Manoil & Rosenbusch, 1982). The kinetics of lac transfer of 1:1 mating mixtures between different donors and Cl22- are shown in Fig. 2. The number of lac recombinants was determined by selecting for recipients ( $\text{spc}^R$ ) and for lac marker transfer on McConkey lac plates containing 30  $\mu\text{g/ml}$  spectinomycin. The transfer blocked mutant in the traI protein could, as expected, not transfer the lac marker. In the light microscope an irreversible, shear resistant association of bacteria could be observed. The same donor bacterium, but this time containing a wild-type F lac plasmid (JC3272F), reached after 90 min about 70% of successful lac transfer. A 1:1 mating mixture between the sex factorless JC3272 and Cl22- was also not able to transfer the lac marker (control) and did not aggregate. With a

transfer blocked *traI* mutant it was therefore possible to accumulate stable wall to wall contacts between as much as 70% of the bacteria present after 90 min of incubation.

For providing another sample for electron microscopy, where more than 50% of the observed bacteria present were actually transferring DNA, the construction of a hfr strain was necessary. As plasmid to be inserted into the *E. coli* chromosome, JCFL39 (*traD*<sup>ts</sup>) was chosen. This F-plasmid in JC3272 (i.e. JC6140) was used by Panicker and Minkley (1985) to demonstrate that DNA transfer occurs during cell surface contact stage by temperature shift experiments. The basic strain JC3272 has a defect in the DNA repair systems responsible for hfr insertion (C. Manoil, pers. comm.) that makes it an ideal host for stable maintenance of chromosome independent F-plasmids.

To be able to induce an hfr-formation between JCFL39 and the bacterial chromosome, the JCFL39 was introduced into the strain WA3110 *lac*<sup>-</sup>. Hfr inserts were selected as described in Materials and Methods. The selected hfr were then recombined by mating to JC3272 in order to have genetic properties of both strains WA3110 *lac*<sup>-</sup> (prototrophy) and JC3272 (*strA*<sup>R</sup>, independent maintenance). The clones were tested for possessing a complete bacterial chromosome with an inserted complete F-plasmid by their ability to be able to transfer all selectable markers more than once. One of them (Hfr1) was chosen and mapped by hfr mapping with AB1157:: pBR325. The mapping is shown in Fig. 3a. The JCFL39-plasmid has been inserted between 0' and 1.7' on the standard *E. coli* K12 map (Fig. 3b). Lac positive recipients collected 30 min after

start of mating were not able to transfer this marker in further matings. Lac positive recipients collected 240 min after start of mating were able to transfer lac in further matings. Therefore the transfer starts at *oriT* and the DNA is transferred in the following order: First F-plasmid coded lac, then the bacterial chromosome starting with leucine and at the end the rest of the F-plasmid containing the *tra*-functions (Fig. 3b). The plasmid is most probably inserted into the chromosome via the  $\gamma\delta$  sequence or one of the IS-elements coding between 0 and 20 kb on the F-plasmid. The insertion locus is very near to that of *hfrH*.

In a 1:1 mating of *Hfr1* with JC5484 at 30°C the kinetics of the lac marker transfer (representing an early marker) of the number of prototroph recombinants (representing late markers) and the ratio of donor to recipient were followed (Fig. 4). From these data we calculate that after 150 min of incubation, about 60% of the bacteria present ( $\text{lac}^+$  recombinants minus prototroph recombinants) are at the state of transferring DNA from donor to recipient. The initiation of transfer and the DNA transfer itself is slower with the *traD<sup>ts</sup>* mutant at the permissive temperature of 30°C than with the wildtype plasmid as can be seen in Fig. 2. when the transfer curve of *Hfr1* x *Cl22<sup>-</sup>::pBR325* is compared with the curve for *JC3272F* x *Cl22<sup>-</sup>*. All transfer efficiencies were determined in nonagitated liquid cultures. Even higher efficiencies can be expected if mating pairs had been collected as a monolayer on an agar surface because they are not all exposed to hydrodynamic forces.

Temperature shift experiments have been considered to be applied on stable mating pairs collected at nonpermissive temperatures and then to initiate transfer by a shift to permissive temperature. The experiments showed that the re-activation of the *traD* function took more than four hours; meanwhile the ratio of donor to recipient was increasing drastically because of unequal growth rates.

#### Electron microscopy of mating pairs

Three samples have been chosen for demonstrating different types of cell to cell contacts in thin sections. First a sex-factorless mating between JC3272 and Cl22<sup>-</sup> after 90 min at 37°C (Fig. 5a, control), second a *traI*<sup>-</sup> transfer blocked mating between JC6296 and Cl22<sup>-</sup> after 90 min at 37°C (Fig. 5b) and third a prolonged mating between Hfr1 and Cl22<sup>-</sup> after 150 min at 30°C (Fig. 5c). Horizontal thin sections through a monolayer of bacteria always show longitudinally cut bacteria, because the axis of the rods is positioned parallel to the agar surface (see Materials and Methods). During incubation on the agar surface before cryofixation the bacteria are still growing exponentially. Under these conditions, Cl22<sup>-</sup> is no more ovoid but has a rod-like appearance. The peripheral "fragmented" nucleoid (defined as ribosome free space, Hobot et al., 1985) is still visible. In average the rods of Cl22<sup>-</sup> have a larger diameter and they contain starch granula. The donor strain JC3272 and its derivatives show a cleft, but confined shape of the nucleoid (Hobot et al., 1985). In these preparations, three morphologically distinct types of contacts are found, which are:

i) septates of dividing bacteria; they are found in all three preparations (in Fig. 5 they are marked as A); ii) juxtapositions of membranes apparently without interaction; they are found in all three preparations (in Fig. 5 they are marked as B); iii) conjugation specific contacts; they are found in transfer-blocked preparations and transferring preparations exclusively (in Fig. 5 they are marked as C). A statistical evaluation of these contacts is given in table II. The conjugation specific contacts are characteristic by the presence of an electron dense (dark) line in between the outer membranes of the conjugating partners. No fusions or visible gaps could be found in serial sections spanning the contact area from top to bottom (Fig. 6). Since sections have a thickness of about 60 nm, smaller structures could be hidden inside or small structures could be masked by superposition. To explore these possibilities, tilt series from  $-60^{\circ}$  to  $+60^{\circ}$  along a horizontal axis positioned within the contact area have been carefully examined. No particular, characteristically defined substructures are found. Figure 7 is a stereopair of micrographs in tilt angles of  $\pm 20^{\circ}$ . Substructures in conjugation contacts that are related to the DNA transfer must be smaller than the resolution of about 50 Å achieved in the thin sections presented.

## DISCUSSION

The stereopictures of contact of DNA transferring pairs (Fig. 7) might be interpreted as shown in the scheme of Fig. 8. Without any doubt, the outer membranes of the two partners are connected in a very tight way. This zone of contact is strongly stained suggesting the presence of a proteinous, glue-like substance in between the two outer membranes. Indeed, the thickness of the contact area is more than twice that of an outer membrane. Throughout the whole contact area no fusion between the inner membranes of the donor and the recipient are visible. In donor as well as in recipient cells there are molecules visible that have a globular domain in the inner membrane and extend a fibrillar domain into the periplasmic gel, but no continuous channel-like structure exists that connects the inner membrane of the donor cell with the inner membrane of the recipient cell. The amount of these structures is about the same in donors and recipient cells. The inner membrane of the donor is not distinguishable by any visible detail from the inner membrane of the recipient. From the aspect on micrographs, we defined the conjugation specific contacts as conjugational junctions. Since DNA is passing through these junctions, a multi elemental mechanism for the DNA-transfer with dimensions smaller than 50 Å has to be supposed.

The membranes of the two partners involved in the conjugation have a completely different set of conjugation specific proteins. The donor has all the proteins that are encoded on the F-plasmid. TraA,L,E,K,B,V,C,W,U,F,Q,H,G are

involved in pili expression, *traN* and *G* are responsible for stabilisation of wall to wall contacts, *traS* and *T* provide the surface exclusion functions and *oriT*, *traM*, *Y*, *D*, *I*, *Z* are responsible for triggering or DNA metabolism during transfer (Ippen-Ihler & Minkley, 1986). The intracellular location (IM, OM or cytoplasm) of these proteins was determined by biochemical methods, but does not allow a definition of their precise function.

The only factors needed for the recipient to be an efficient partner are *ompA* and a heptose containing lipopolysaccharide in the outer membrane (Manoil & Rosenbusch, 1982). Probably some further indispensable factors are needed in the recipient to enable DNA-transfer that have not yet been tested (e.g. sugar uptake systems: see later in Discussion). The possibility that proteins are exported from the donor into the recipient during mating has been proposed, but was never substantiated by convincing experiments.

We define the outer membrane plus the peptidoglycan layer as bacterial cell wall. According to our pictures, the conjugation specific junctions are intensive contacts between the cell walls of donor and recipient. When the curves for prototrophy and lac-transfer in Fig. 4 are compared at  $t = 150$  min, 6 among 10 conjugation specific junctions (% lac-transfer - % prototrophs) between bacteria in the mixture of Hfr1 x Cl22 should actually transfer DNA. Therefore, the probability is high that what we see are active junctions. Previous experiments that were done with undefined cultures and without orientation into a monolayer result in a probability which is below any realistic number (Gross &

Caro, 1966; Schreil & Christensen, 1968). The three types (Fig. 5, Table II) of interbacterial contacts had indeed never been shown before. The contacts of the type C are clearly related to conjugation. The thickness of a thin section ( 700 Å) cannot show the total area of the conjugation specific junction. Therefore serial sections have been made and the junctions were screened throughout for more details, but they had a uniform aspect. There is no visible difference between the junctions of arrested and DNA-transferring matings, therefore the components of the transfer mechanism must be smaller than what can be observed by thin sections in electron micrographs. Pili are too small structures ( 20 Å internal tube diameter) to be seen in such sections (Folkhard et al., 1979) and we cannot exclude the hypothesis that remains of pili do form a pore reaching from the inner membrane of the donor to the inner membrane of the recipient.

Our data confirm the models formulated about the mechanism of bacterial conjugation published earlier in reviews (Willets & Wilkins, 1984; Willets & Skurray, 1980). In the proposed models, the DNA is transferred by the help of plasma bridge resulting from a fusion of the inner and outer membranes of the two partners. We cannot support this part of the working hypothesis, therefore we try to refine the existing mechanism for the DNA transfer to make it also consistent with our new electron microscopic data. TraD may form a porelike structure reaching through the inner membrane and the cell wall of the donor. The DNA is driven by a probably membrane bound helicase (traI protein) through the pore formed by traD (Willets & Wilkins, 1984) into the peri-

periplasmic gel of the recipient. In the recipient there is no F-plasmid coded protein in the inner membrane such as traD. In this detail the uptake mechanism has to be different from the one proposed (Willems & Wilkins, 1984). A conceivable mechanism would be a similar uptake of the DNA into the recipient like the infection of bacteriophage  $\lambda$  (Scandella & Arber, 1974, 1976; Elliot & Arber, 1978; Erni et al., 1987), where DNA is injected into the periplasmic gel and then transported through the inner membrane by the sugar uptake system for manose (ptsM). In analogy, any pore-former connected with the metabolism of nutrients could be "missused" in conjugation for DNA uptake. Such a principle has probably not yet been detected because these metabolic systems are ubiquitous. However, there are some hints in literature that sugar metabolism is important for conjugation, e.g. the uptake of RNA during R17 infection is pili mediated and dependent on the sugar source on which the bacteria had been grown (O'Callaghan et al., 1973; Danziger & Paranchych, 1978). The uptake of RNA grown on glucose (ptsG expressed) could not be prevented by sugar analogue inhibitors as it was possible for bacteria grown on glycerol. It might also be possible that DNA is taken up into the recipient during conjugation through ion transport systems. Increased concentrations of  $\text{Na}^+$  or  $\text{K}^+$  in the medium during conjugation facilitate the mating efficiencies (Singelton, 1983).

It has been shown already that the whole mechanism of conjugation goes through a series of defined intermediary stages. In all recently presented models (Willems & Skurray, 1980; Panicker & Minkley, 1985), the role of pili is clearly

the mediation of contact between donor and recipient and the DNA transfer is performed at the state of stabilised wall to wall contact. The hypothesis that DNA is transferred through remains of pili is highly improbable since *traD* mutants do not transfer DNA and stabilised mating pairs are accumulated. Mating pairs in stabilised wall to wall contact can be collected by a *traD*<sup>ts</sup> mutant at non permissive temperature and freed of pili by SDS treatment. After the stable mating pairs were shifted to the permissive temperature, where *traD* is functional, they were able to transfer DNA (Panicker & Minkley, 1985). Therefore, the pilus seems to be the organ that provides the first contact between extended pairs and mediates closer contact by retraction (a mechanism that is not yet fully understood). In addition, the pilus has possibly the function of transmitting somehow a signal through the inside after successful contact formation which might trigger the DNA transfer metabolism (Brinton, 1974; Datte et al., 1977; Helmuth & Achtman, 1978; Frost et al., 1984 and L. Frost., pers. comm.). To be able to perform these functions the tip and the base of pili seem to be composed of different proteins than pilin (Frost et al., 1986; Worobec et al., 1986). The protein sitting at the base of pili is suspected to be *traM* which is involved in triggering the DNA metabolism (Achtman et al., 1971; Willets & Wilkins, 1984). The conjugation specific junctions that were shown in this paper and the DNA transfer seems to be the result of an ordered cascade of regulatory steps that are as yet only poorly understood.

We have drawn a mechanism for bacterial conjugation (Fig. 9) that is a refinement of the models presented by Willets & Wilkins (1980) and by Panicker & Minkley (1985) containing the improvements deduced from micrographs of conjugational junctions.

CHART I

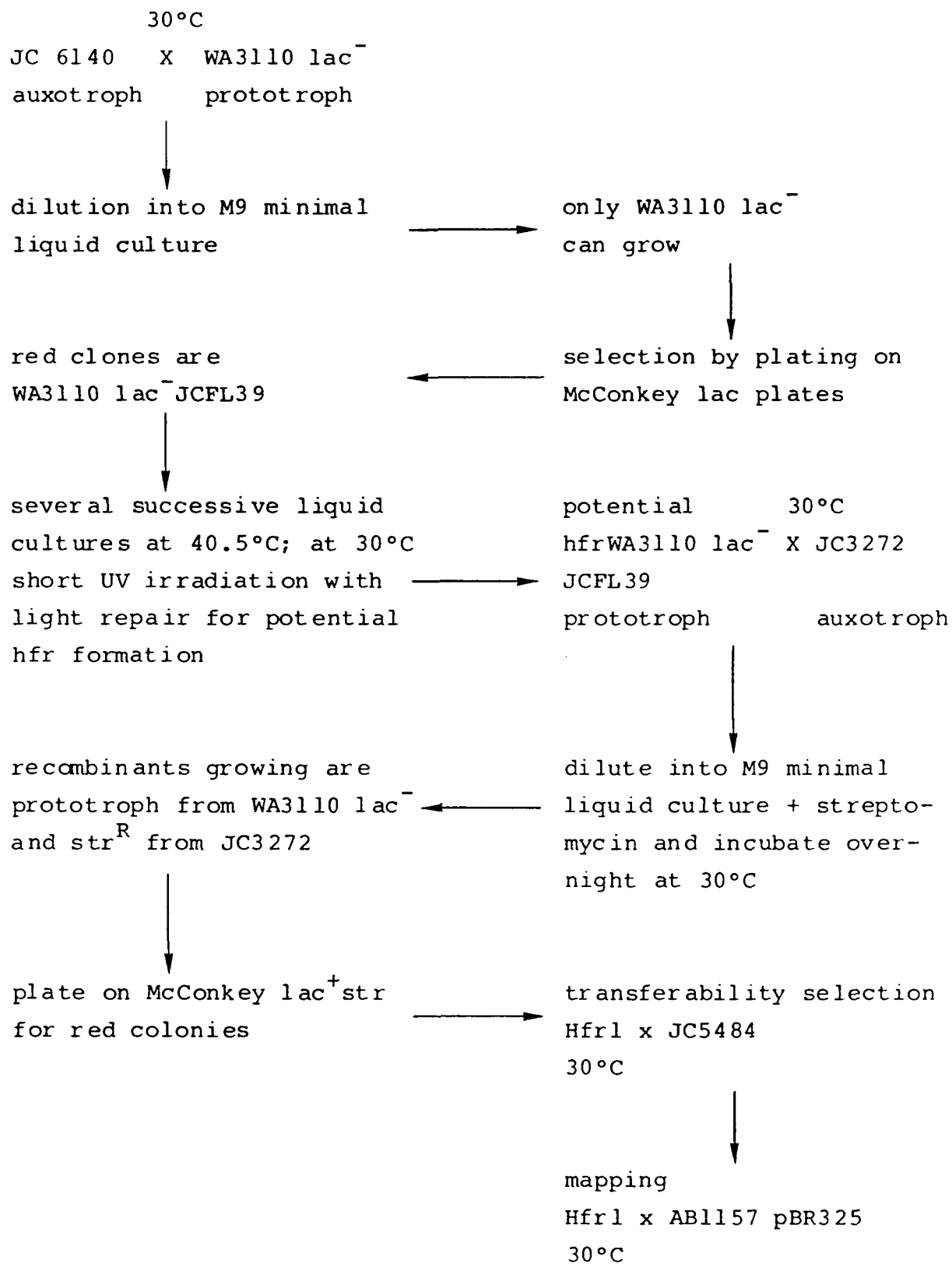


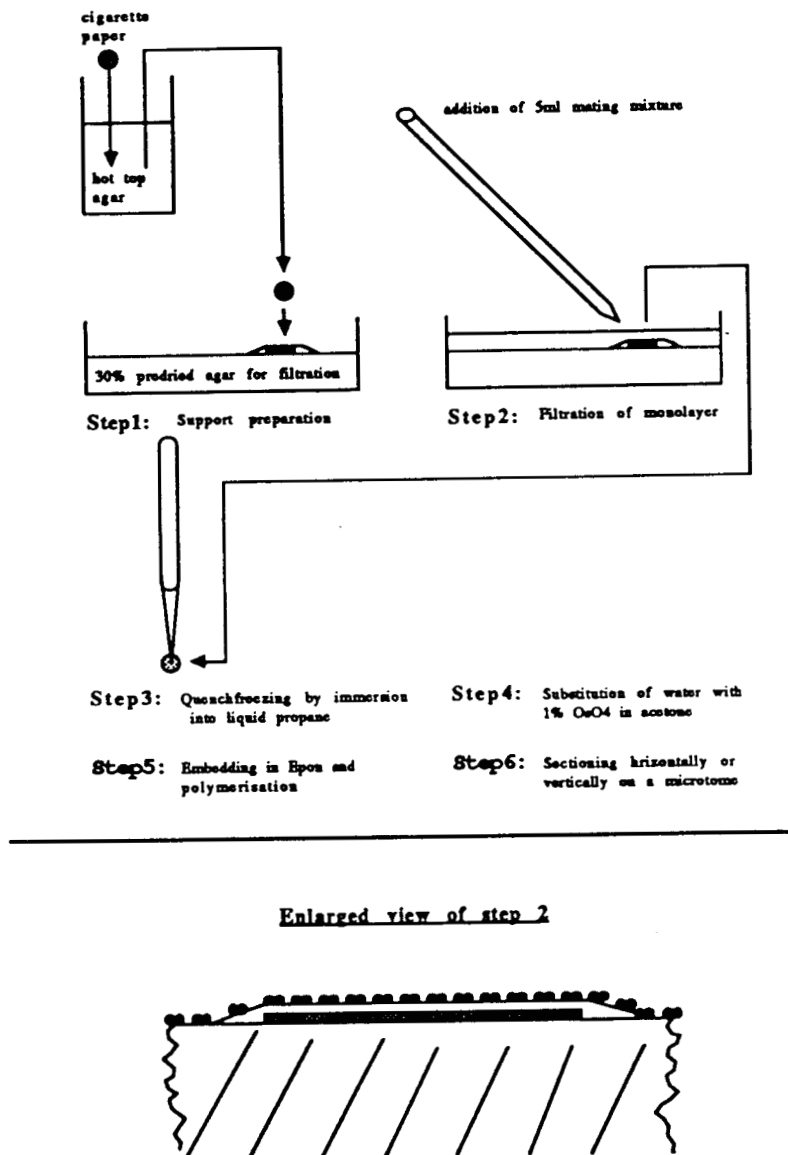
TABLE I

Strain	Markers	For F'plasmid	ompA	Origin
JC3272	his, trp, lys, tsx gal, malA <sup>-</sup> , lac X74 strA <sup>R</sup>	-	+	Achtman et al. (1971)
JC5484	his, trp, ton, tsx lac X74, spc <sup>R</sup>	-	+	Achtman et al. (1971)
JC6296	derivative of IC3272	ICFL41:lac, traI <sup>-</sup>	+	Achtman et al. (1971)
JC6140	derivative of IC3272	ICFL39:lac, traDts	+	Achtman et al. (1971)
Hfr1	derivative WA3110lac <sup>-</sup> (K12) crossed with IC3272	ICFL39:lac, traDts incorporated between 0' and 1.7' on <u>E. coli</u> std map	+	Construct WA3113lac <sup>-</sup> from W. Arber's collection (Biocenter UNI Basel)
JC3272F	derivative of IC3272	F42 lacI3:lac, Kam <sup>R</sup>	+	Construct by mating
C122	met, arg, try, ade	-	+	British culture collection
C122 <sup>-</sup>	derivative of C122 lac <sup>-</sup> spc <sup>R</sup>	-	+	Construct by UV mutagenesis and selection on spc plates.
AB1157::	derivative of AB1157			Construct by transformation of AB1157 (Howard and Theriot, 1966).
pBR325	Amp <sup>R</sup> Tet <sup>R</sup> Cam <sup>R</sup>	-	+	

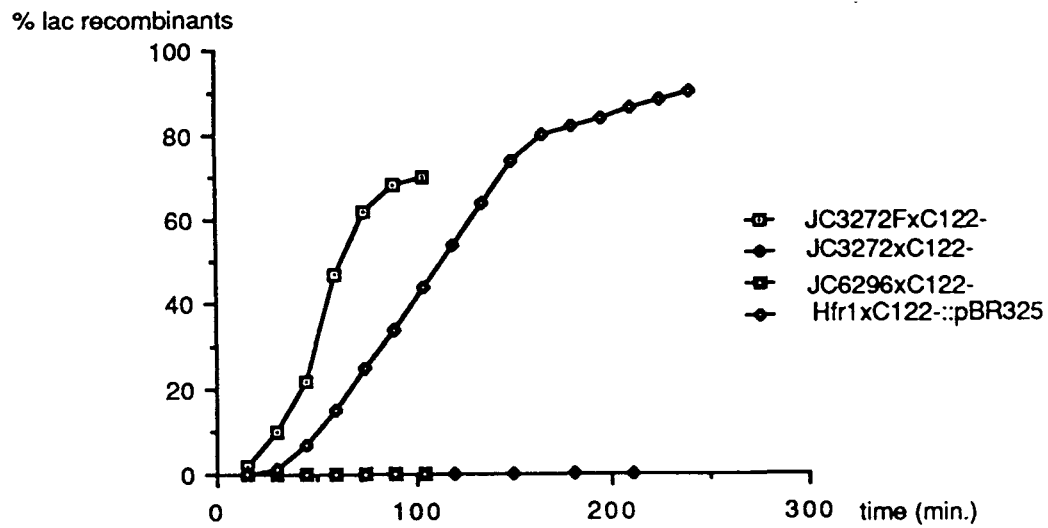
TABLE II

Kinds of contacts between bacteria (N = 100) in monolayers.

Monolayer	Septate	Inactive	Conjugation
	formation	yuxtapositions	spec. junctions
	A	B	C
JC3272 x C122	18	106	0
JC6296 x C122	12	84	71
Hfr1 x C122	11	44	70

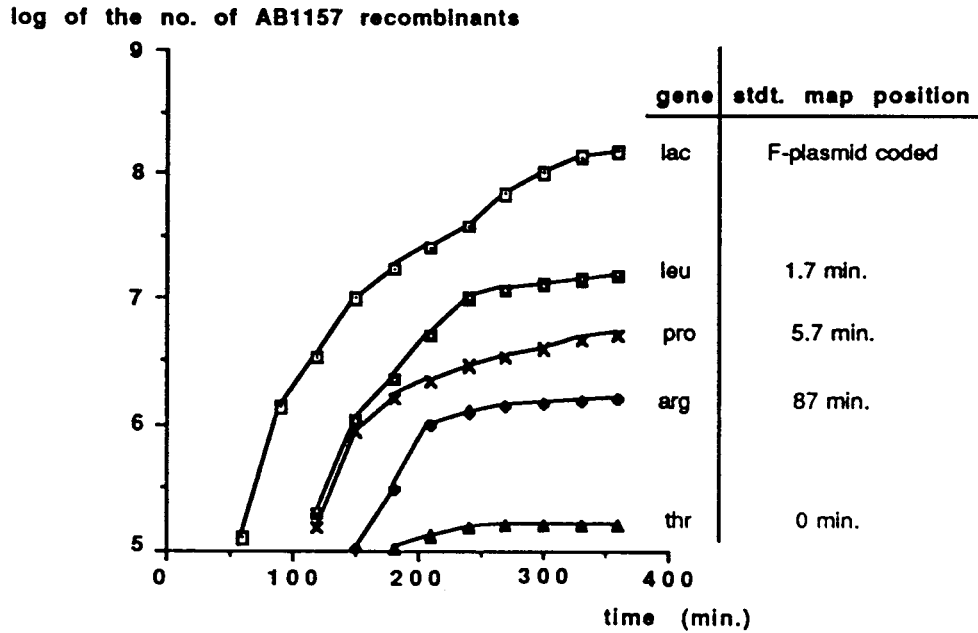


**Fig.1:** A scemtical representation of how a monolayer of conjugating bacteria is prepared for electron microscopy. The single steps are further described in materials and methods.

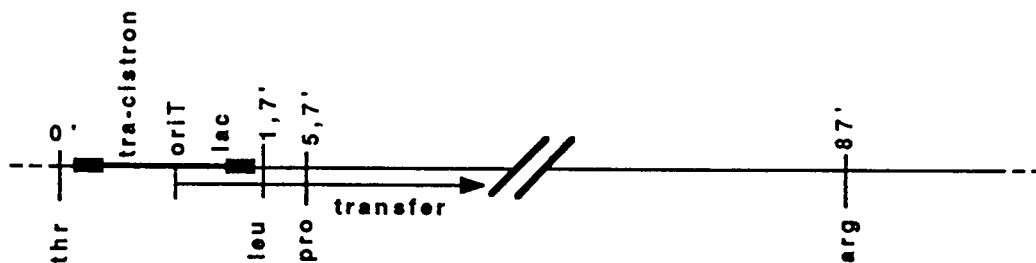


**Fig.2:** The kinetics of lac-transfer of the different 1:1 mating mixtures are plotted. JC 3272 (no F-plasmid) and JC 6296 (tra I mutant) are unable to transfer the F-plasmid coded lac marker. JC 3272F mixed with C122- is referred to as standard. The Hfr 1 mixed with C122- shows a delayed marker transfer due to the ts-modification in tra D.

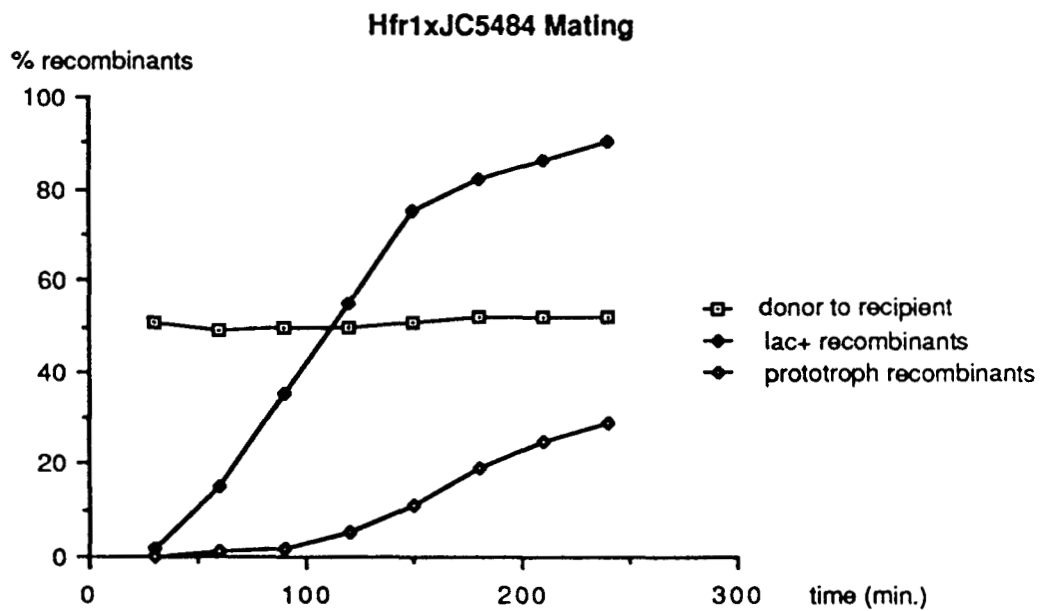
a)



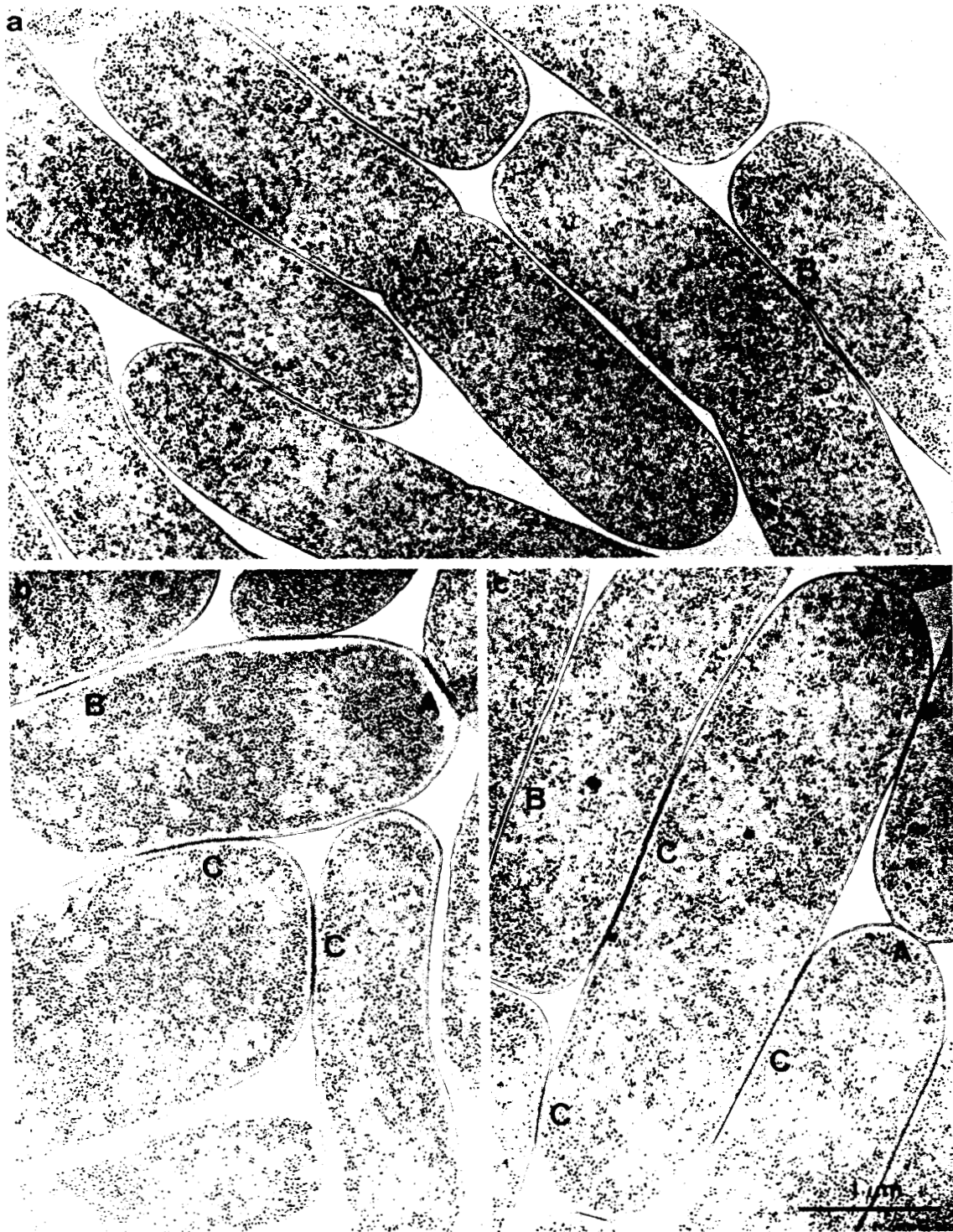
b)



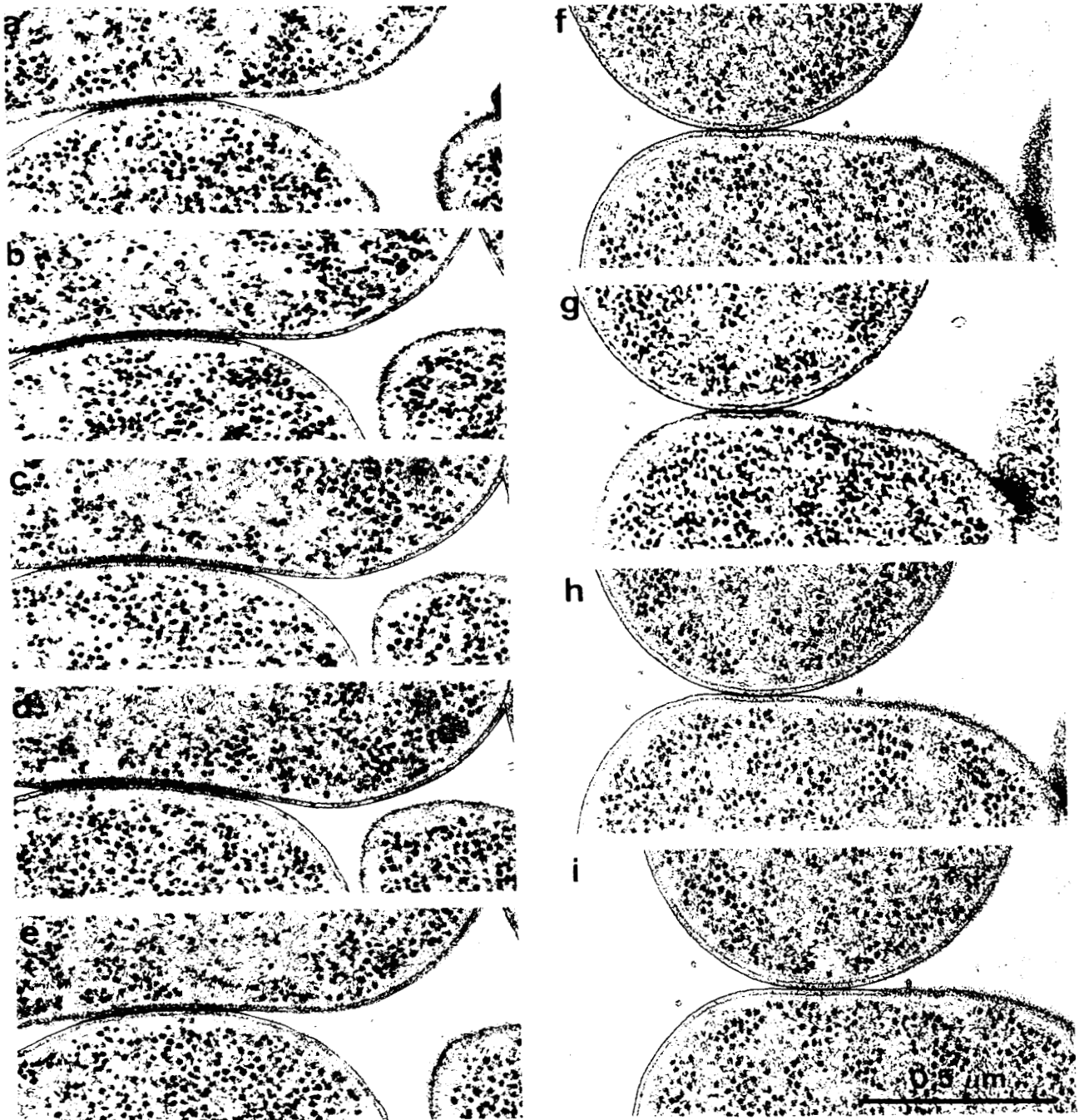
**Fig.3:** A physiological map of Hfr 1 was made to show where JC FL 39 is integrated into the bacterial genome. In (a) the kinetics of the transfer of the different markers by Hfr 1 into AB 1157 is plotted. A linear map based on the information from (a) is drawn in (b). The position of ori T and the direction of transfer was determined by the finding that only AB 1157 recombinants that have received the thr marker were able to act as donors in further matings.



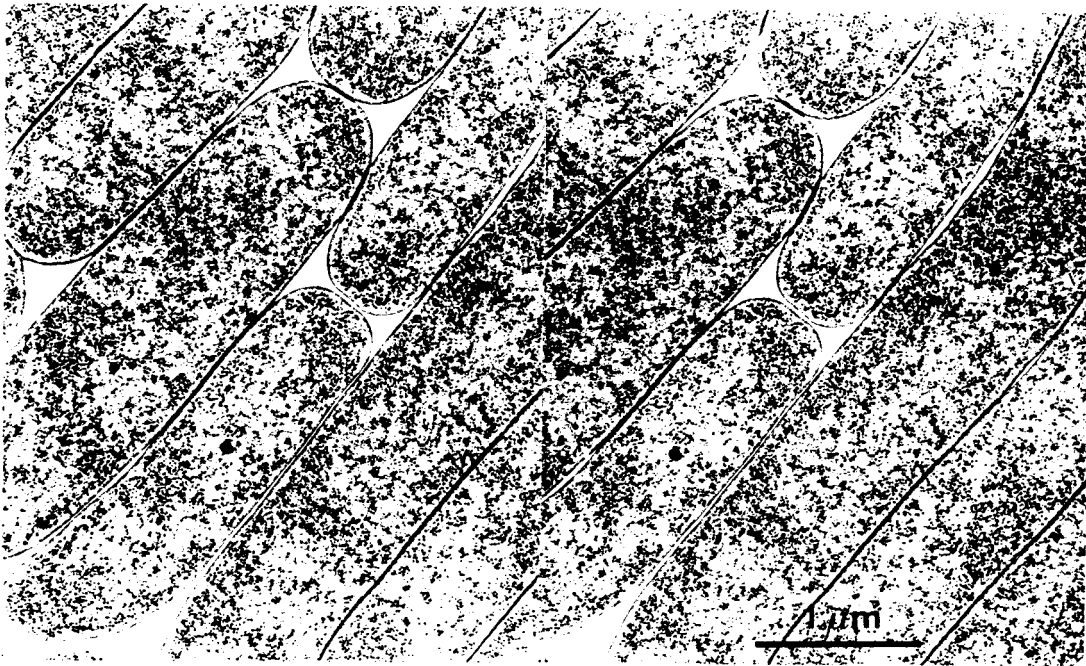
**Fig.4:** The kinetics of the early transferred lac marker and the full prototrophy (representing a late marker) in a 1:1 mating mixture between Hfr 1 and JC 5484 is plotted. The donor to recipient ratio was maintained constant during the time monitored, because the two strains have a very similar growth rate.



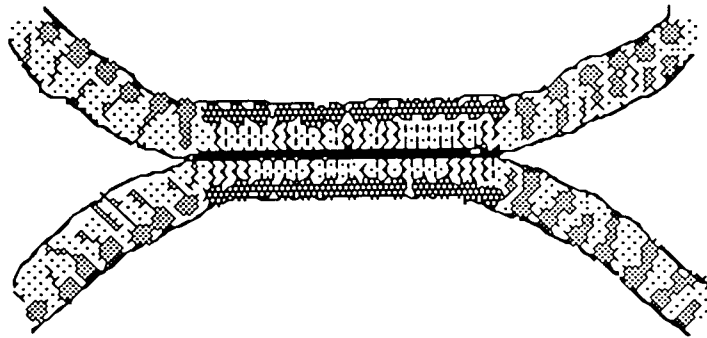
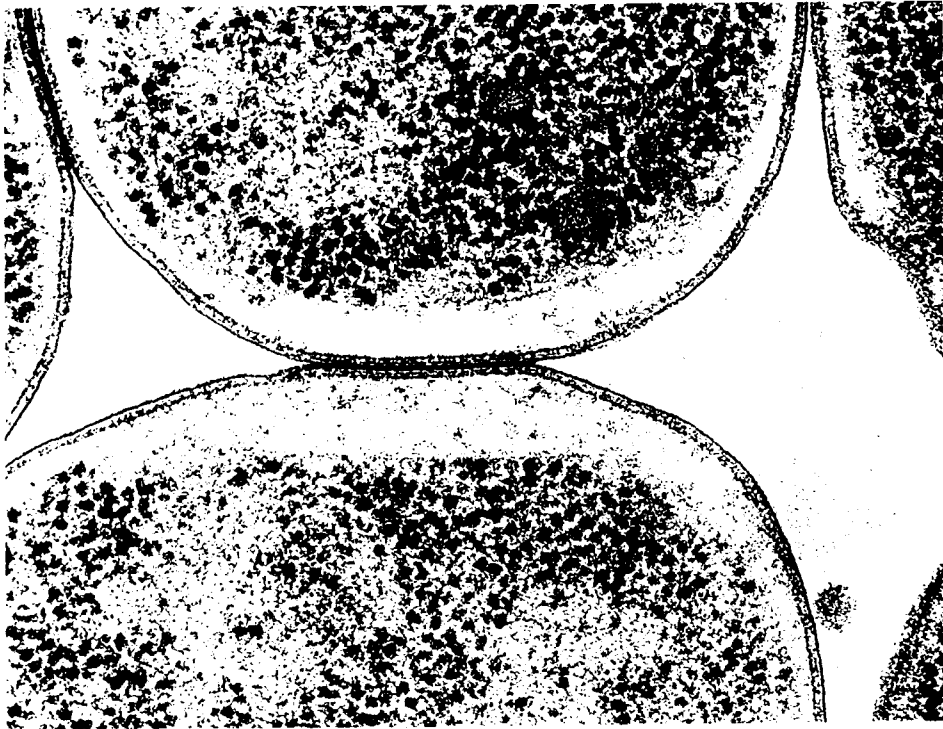
**Fig.5:** Micrographs of horizontally sectioned monolayers of JC3272 (no F-plasmid) mixed with C122- (a), JC6296 mixed with C122- (b) and Hfr 1 mixed with C122-. In the control mating without F-plasmid (a) there are only septate formations (marked with A) and inactive iuxtapositions (marked with B) between bacteria visible. The transfer blocked mating (b) and the actively transferring mating (c) show in addition conjugational junctions (marked with C). A statistical evaluation is given in table 2.



**Fig.6:** Serial thin section through conjugation specific junctions are shown. (a) to (e) is a horizontally sectioned serie and (f) to (i) a vertically sectioned serie of Hfr 1 mixed with C122-. There are no cytoplasmic bridges nor any pores visible over the whole range of the junctions.

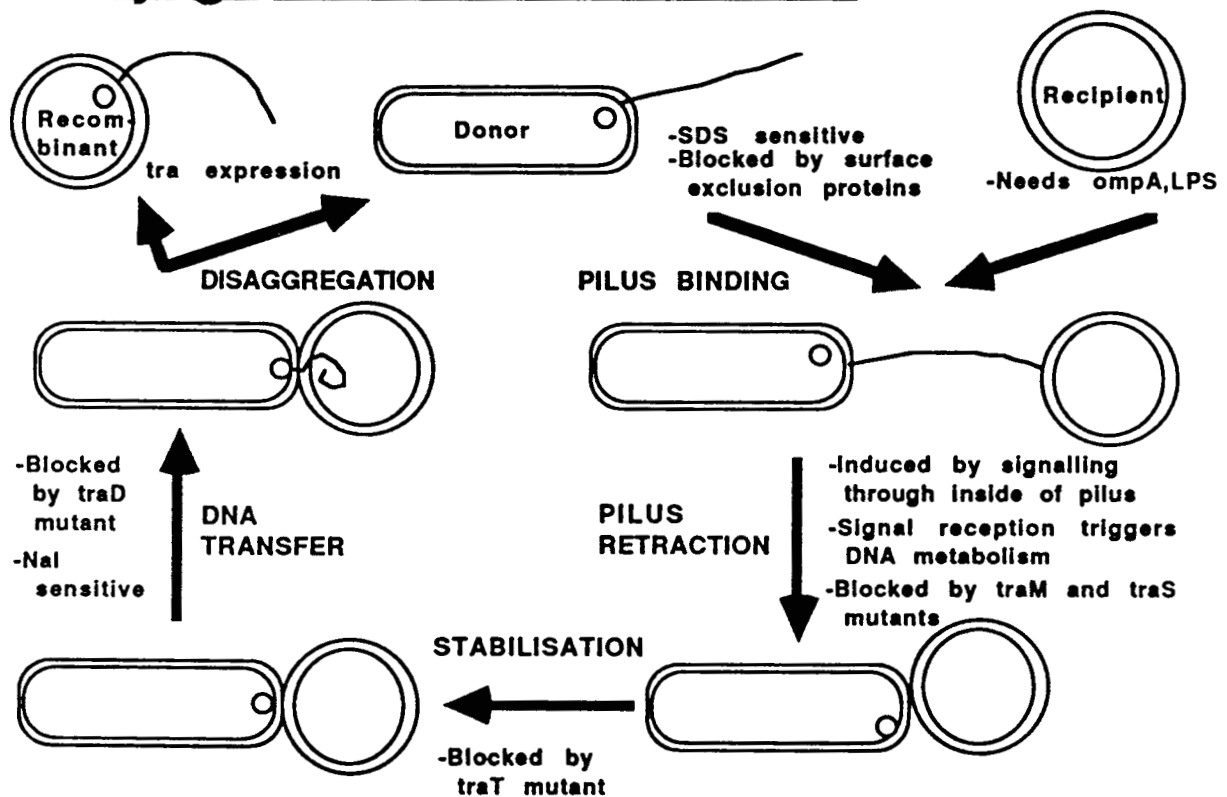


**Fig.7:** Stereo effect micrograph of a mating between Hfr 1 and C122-. Tilt angles are  $-7^{\circ}$  on the left half and  $+7^{\circ}$  on the right half. The zero position was chosen as  $90^{\circ}$  to the sections surface plane. The depth is about the middle of of the conjugational junctions (third section from the top of the junction). The should be looked at with a stereo viewer. ALso with this technique no cytoplasmic bridge or pore was visible throughout the whole conjugational junction.



**Fig.8:** Shows a closeup of a conjugational junction between Hfr 1 and C122-. From several similar micrographs we deduced the information for the model drawing in the lower half of the figure.

## Conjugational mechanism



**Fig.9:** We have drawn a refined version of the conjugational mechanism of Willets and Wilkins that contains the new information about conjugational junctions.

## REFERENCES

- Achtman, M., N. Willets, and A.J. Clark. 1971. Beginning a genetic analysis of conjugational transfer determined by the F factor in Escherichia coli by isolation and characterization of transfer-deficient mutants. J. Bacteriol. 106:529-538.
- Armstrong, G.D., L.S. Frost, P.A. Sastry, and W. Paranchych. 1980. Comparative biochemical studies on F and EDP208 conjugative pili. J. Bacteriol. 141:333-341.
- Brinton, C.C. Jr. 1971. The properties of sex pili, the viral nature of "conjugal" genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. Crit. Rev. Microbiol. 1:105-160.
- Curtiss, R. 1969. Bacterial conjugation. Ann. Rev. Microbiol. 23:69-136.
- Danziger, R., and W. Paranchych. 1970. Stages in R17 infection. III. Energy requirements for the F pili mediated eclipse of viral infectivity. Virology 40:554-564.
- Date, T., M. Inuzuka, and M. Tomoeda. 1977. Purification and characterization of F pili from Escherichia coli. Biochemistry 16:5579-5585.
- Elliott, J. and Arber, W. 1978. E. coli K-12 pel mutants, which block phage  $\lambda$  DNA injection, coincide with ptsM, which determines a component of a sugar transport system. Mol. gen. Genet. 161:1-8.
- Erni, B., Zanolari, B. and Kocher, H.P. 1987. The mannose permease of Escherichia coli consists of three different proteins.

- Folkhard, W., Leonard, K.R., Malsey, S., Marvin, D.A., Dubochet, J., Engel, A., Achtman, M. and Helmuth, R. 1979. X-ray diffraction and electron microscope studies on the structure of bacterial F pili. *J. Mol. Biol.* 130:145-160
- Frost, L.S., J.S. Lee, D.G. Scraba and W. Paranchych. 1986. Two monoclonal antibodies specific for different epitopes within the amino-terminal region of F pilin. *J. Bacteriol.* 168:192-198.
- Frost, L.S., W. Paranchych, and N.S. Willetts. 1984. DNA sequence of the F traLE region that includes the gene for F pilin. *J. Bacteriol.* 160:395-401.
- Gross, J.D., and L.G. Caro. 1966. DNA transfer in bacterial conjugation. *J. Mol. Biol.* 16:269-284.
- Helmuth, R., and M. Achtman. 1978. Cell-cell interactions in conjugating Escherichia coli: Purification of F pili with biological activity. *Proc. Natl. Acad. Sci. USA* 75: 1237-1241.
- Hobot, J.A., E. Carlemalm, W. Villiger, and E. Kellenberger. 1984. Periplasmic gel: New concept resulting from the reinvestigation of bacterial cell envelope ultrastructure by new methods. *J. Bacteriol.* 160:143-152.
- Hobot, J.A., W. Villiger, J. Escaig, M. Maeder, A. Ryter, and E. Kellenberger. 1985. Shape and fine structure of nucleoids observed on sections of ultrarapidly frozen and cryosubstituted bacteria. *J. Bacteriol.* 162:960-971.
- Howard-Flanders, P., and L. Theviot. 1966. DNA repair and recombination defective mutants of E. coli K12. *Genetics* 53:1137.

- Ippen-Ihler, K.A., Minkley, E.G., Jr. 1986. The conjugation system of  $F_1$  the fertility factor of E. coli. Ann. Rev. Genet. 20:593-624.
- Lieb, M., J.J. Weigle, and E. Kellenberger. 1955. A study of hybrids between two strains of E. coli. J. Bacteriol. 69:468-471.
- Manoil, C., and J.P. Rosenbusch. 1982. Conjugation deficient mutants of E. coli distinguish classes of functions of the outer membrane OmpA protein. Mol. Gen. Genet. 187:148-156.
- Marvin, D.A., and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. 33:172-209.
- O'Callaghan, R.J., L. Bundy, R. Bradley, and W. Paranchych. 1973. Unusual arsenate poisoning of the F pili of Escherichia coli. J. Bacteriol. 115:76-81.
- Ou, J.T., and T.F. Anderson. 1970. Role of pili in conjugation. J. Bacteriol. 102:648-654.
- Panicker, M.M., and E.G. Minkley, Jr. 1985. DNA transfer occurs during a cell surface contact stage of F-sex factor mediated bacterial conjugation. J. Bacteriol. 162:584-590.
- Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.
- Scandella, D. and Arber, W. 1974. An Escherichia coli mutant which inhibits the injection of phage  $\lambda$  DNA. Virology 58:504-513.
- Scandella, D. and Arber, W. 1976. Phage  $\lambda$  DNA injection into

- Escherichia coli pel<sup>-</sup> mutants is restored by mutations in phage genes V or H. *Virology* 69:206-215.
- Schreil, W.H., and R.J. Christensen. Bacterial conjugation: Electron microscope observations on thin sections. 1968. *Proc. Natl. Acad. Sci. USA* 59:1152-1157.
- Singelton, P. 1983. Zeta-potential: A determination factor in F-type mating. *FEMS Microbiol. Lett.* 20:151-153.
- Willets, N., and R. Skurray. 1980. The conjugation system of F-like plasmids. *Ann. Rev. Genet.* 14:41-76.
- Willets, N., and B. Wilkins. 1984. Processing of plasmid DNA during bacterial conjugation. *Microbiol. Rev.* 48:24-41.
- Worobec, E.A., L.S. Frost, P. Pieroni, G.D. Armstrong, R.S. Hodges, J.M.R. Parker, B.B. Finlay, and W. Paranchych. 1986. Location of the antigenic determinants of conjugative F-like pili. *J. Bacteriol.* 167:660-665.